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REMARKS

Claims 31-78 are pending in the application. Claims 75-78 are withdrawn. Reconsideration of the present application is respectfully requested.

Rejection under 35 USC Section 103

The Examiner has rejected claims 31-74 as being obvious over Romanczyk, Jr. (US 5,554,645; hereinafter referred to as 'Romanczyk') in view of Wideman *et al.* (US 6,127,421; hereinafter referred to as 'Wideman') on the ground that it would have been obvious to modify the food composition of Romanczyk by adding L-arginine because both references use their respective compounds for anti-tumor purposes. Applicants respectfully traverse the rejection.

The primary focus and teaching of Wideman is the use of L-arginine in eggs for treatment/prevention of pulmonary hypertension syndrome in birds (*see,* entire patent). Regarding the teachings in Wideman of anti-tumor utilities, the Examiner refers to a background citation therein of Taylor *et al.* 1992 and the statement that dietary administration of L-arginine to chicken reduces tumor growth. However, Applicants submit that as of the effective filing date of the above Application, the knowledge in the art as to the anti-tumor/cancer effect of dietary arginine supplementation in mammals was highly controversial and various contradictory reports existed in the field. For example, see, Yeatman T. J. *et al.* Depletion of Dietary Arginine Inhibits Growth of Metastatic Tumor, *Arch. Surg.* 1991, 126(11):1376-82 (Attachment No. 1, relevant text is underlined). Using a mouse (mammalian) model, Yeatman showed that dietary arginine <u>depletion</u> inhibited the growth of liver metastases of colorectal cancer cells. Thus, Yeatman teaches away from the use of dietary arginine supplementation to inhibit tumor/cancer growth, therefore a person of skill in the art would <u>not</u> have been motivated to modify the food of Romanczyk by adding L-arginine with any reasonable expectation of success that both the polyphenol and L-arginine would have had anti-tumor effects.

Applicants further point out that Wideman's subjects are birds, while Romanczyk's subjects are mammals. Thus, in view of the facts that (i) the anti-tumor effects of arginine were uncertain, and (ii) prior art taught different subjects, a person skilled in the art would not have been motivated to use L-arginine of Wideman (*i.e.*, Taylor *et al.*) to modify the food composition of Romanczyk and reasonably expect to succeed.

Additionally, neither Romanczyk nor Wideman (alone or in combination) teach or suggest the non-chocolate food composition containing polyphenols and L-arginine "in an *amount effective* to induce a physiological increase in nitric oxide" (emphasis added). The Examiner has not given weight to the effective amount limitation in Applicants' claims as he appears to believe that it is not limiting as it relates to intended use. Applicants' respectfully submit that the effective amount limitation is a functional limitation which (together with a numerical limitation for the amount of L-arginine) structurally defines the scope of the claims, *i.e.*, it defines the amount of the compounds required to be present in the non-chocolate composition. Without any teaching or suggestion to this effect, a person skilled in the art would not have been motivated to prepare the non-chocolate composition of claims 31-74 and have a reasonable expectation of success at achieving the composition capable of increasing NO levels.

In view of the above remarks, withdrawal of the rejection is respectfully requested.

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CONCLUSION

In view of the above remarks, Applicants believe that the application is now in condition for allowance. A notice to that effect is respectfully requested.

Date: May 9, 2007

Respectfully submitted,

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Depletion of Dietary Arginine Inhibits Growth of Metastatic Tumor

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• The effects of dietary arginine on the growth of a murine colon tumor metastatic to the liver were examined in a model of advanced neoplastic disease. Tumor growth was influenced by arginine both in vivo and in vitro. An arginine-supplemented diet stimulated tumor growth by 55% compared with controls. Conversely, an arginine-depleted diet inhibited tumor growth by 78% compared with controls. In vitro culture of both murine and human colon tumor cells confirmed that arginine was necessary for cell growth. Flow-cytometric analysis using propidium iodide and bromodeoxyuridine suggested that colon tumor cells cultured without arginine enter a quiescent S phase and depend on arginine for further growth and cell cycle progression. The potential roles for selective dietary arginine modulation in patients with cancer with advanced disease are discussed.

(*Arch Surg.* 1991;126:1376-1382)

The influence of arginine on tumor growth and host nutrition has been investigated for more than 70 years.[1] Despite these efforts, the effect of dietary arginine on tumor growth has not been clearly elucidated. In some systems, arginine has been shown to stimulate tumor growth,[2-5] while in other models, inhibition of tumor growth was noted.[5-8] This apparent paradox may be explained by the concept that in vivo tumor growth is influenced by multiple, competing factors.

It has been postulated that tumor growth is a dynamic process involving a "predator-prey" competition between immunocompetent cells and neoplastic cells in which growth is the vector sum of cell destruction and cell proliferation.[9] In this model, tumor progression depends on the capacity of the immune system to recognize and destroy neoplastic cells—a capacity that may be related to the degree of tumor immunogenicity. Recent reports have suggested that arginine-supplementation may suppress the growth of immunogenic tumors because of host immunostimulatory effects; however, these growth-inhibiting effects were not seen with tumors that were weakly immunogenic. Growth may occur in this system because weakly immunogenic tumor cells escape recognition and destruction by otherwise effective tumor-directed immune responses.[5,10-12]

Using a weakly immunogenic colon cancer model, we determined the effects of arginine on tumor growth. Because arginine is considered essential for in vitro cell culture of both normal and neoplastic cells, we hypothesized that in vivo tumor propagation may depend on dietary arginine and that arginine depletion could inhibit tumor growth. Likewise, because immune defenses directed against weakly immunogenic tumor may not effectively compete with cell proliferation, arginine supplementation might enhance tumor growth.

We chose a model of experimental liver metastasis that simulates a state of advanced neoplastic disease in which nutritional deficits may become clinically relevant. The effects of both dietary arginine supplementation and dietary arginine depletion on the subsequent in vivo growth of liver metastases were examined. Similarly, the effects of arginine depletion and repletion on the propagation of these tumor cells in vitro were investigated.

MATERIALS AND METHODS

Animals

Six- to eight-week-old BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Me) and housed in the Department of Pathology, Tumor Biology Mouse Colony, University of Florida, Gainesville. Mice had free access to solid chow and water and five mice were housed per cage. The mice were age, weight, and sex matched for each experiment.

Cell Lines and Routine Culture Conditions

Cell line CT-26 was originally derived from a chemically induced primary, undifferentiated murine colorectal adenocarcinoma and was syngeneic with the BALB/c murine strain. The cells are known to be weakly immunogenic (as measured with challenge and rechallenge experiments) but highly tumorigenic.[13] Cells were routinely cultured in vitro as a monolayer at 37°C in a humidified incubator containing 7% carbon dioxide in air. Cells were grown in minimal essential medium (Grand Island Biologicals, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Grand Island Biologicals) at 6×10^5 cells per 10 mL. Near confluence, after 4 days of growth, cell monolayers were detached from the Petri dish (No. 3100,

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Presented at the 44th Annual Cancer Symposium of the Society of Surgical Oncology, Orlando, Fla, March 26, 1991.

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Costar, Cambridge, Mass) after a 3-minute incubation at room temperature with 0.7-mmol/L ethylenediaminetetraacetic acid in phosphate-buffered saline, which did not contain calcium or magnesium, supplemented with 0.6-mmol/L glucose and subcultured in fresh medium. Cell viability was determined with trypan blue dye exclusion using a hemocytometer.

Human colon adenocarcinoma cells (HT-29) were obtained from the American Type Tissue Culture Collection (Rockville, Md) and cultured as a monolayer (applying standard conditions explained above) in RPMI medium (Grand Island Biologicals) with 10% fetal bovine serum.

Experimental Metastasis Assay

Experimental liver metastases were produced in vivo using intrasplenic injection. Mice were anesthetized before intrasplenic injection with an intraperitoneal injection of 3 mg of ketamine hydrochloride and 0.03 mg of acepromazine maleate in phosphate-buffered saline. A suspension of 1.25×10^5 to 2.5×10^5 tumor cells in 0.5 mL of phosphate-buffered saline was injected into the inferior splenic pole over approximately 1 minute using a controlled-rate infusion syringe pump (No. 355, Sage Inc, Cambridge, Mass). A small hemoclip was then applied to the inferior splenic pole to prevent hemorrhage and back-diffusion of tumor cells into the free peritoneal cavity. Surgical incisions were closed with metal clips. Splenectomy was not performed. Mice underwent necropsy on day 14, and the extent of hepatic and other metastases was recorded. Mice received an intravenous injection of 10% india ink before necropsy to aid in detection of hepatic metastases. Livers were excised and immediately weighed.

Murine Diets

Standard Chow.—To examine the effects of supplemental arginine, mice were fed with standard solid mouse chow (23.4% protein and 1.38% arginine) ad libitum and were randomly assigned to one of two groups. Mice were permitted to drink water supplemented with 1% arginine hydrochloride or 1.7% (isonitrogenous) glycine ad libitum. Mice were administered supplemented water 7 days before tumor cell inoculation. Solid chow and water intake were monitored in each group.

Amino Acid–Defined Diets.—To examine the effects of dietary arginine depletion, mice were randomly assigned to be fed ad libitum one of two solid chow, amino acid–defined diets: standard-content, arginine-repleted diet (1.2% arginine) or arginine-depleted diet (no arginine). The arginine-depleted diet provided only 0.32% less nitrogen than the arginine-repleted diet. Water without any additives was administered ad libitum. These specific diets were prepared by Teklad Research Diets (Madison, Wis). Specific dietary formulas are listed in Table 1. All mice were placed on the appropriate diet 7 days before tumor cell injections. Solid chow and water intake were monitored for each group.

Culture Conditions Before DNA Analysis

Select-amine kits (Grand Island Biologicals) were used to formulate arginine-depleted or arginine-repleted minimal essential medium and RPMI medium. In vitro tumor growth was analyzed with flow cytometry after culture of 5×10^5 CT-26 tumor cells in 10 mL of minimal essential medium or 5×10^5 HT-29 tumor cells in 10 mL of RPMI. CT-26 cells were cultured without or with (0.013% [0.59 mmol/L]) supplemented arginine. HT-29 cells were cultured in multiple arginine concentrations ranging from none to 1.14 mmol/L. Cultures were harvested after 4 days.

Flow Cytometry

DNA Analysis With Propidium Iodide.—After culture in which specific media conditions were applied, cells were harvested and counted using a hemocytometer. Cells were then fixed in 70% ethanol solution and treated with 10 μg of propidium iodide (PI; Sigma Chemical Corp, St Louis, Mo), per milliliter of solution, 0.5% polysorbate 20, and 400 U ribonuclease I (Sigma)

Table 1.—Composition of Amino Acids in Arginine-Repleted, Defined Diet

Amino Acid	Amount, g/kg
Alanine	3.5
Arginine*	12.1
Asparagine	6.0
Aspartic acid	3.5
Cystine	3.5
Glutamic acid	40.0
Glycine	23.3
Histidine	4.5
Isoleucine	8.2
Leucine	11.1
Lysine	18.0
Methionine	8.2
Phenylalanine	7.5
Proline	3.5
Serine	3.5
Threonine	8.2
Tryptophan	1.8
Tyrosine	5.0
Valine	8.2

*Omitted in composition of arginine-depleted diet.

before flow cytometric analysis using an argon laser (FAC-STAR, Becton-Dickinson, Oxnard, Calif) with an excitation wavelength of 488 nm and a measured emission wavelength of 515±20 nm. Data were collected and analyzed using a computer program (Consort 30, Becton-Dickinson). DNA histograms were used to perform cell cycle analysis. The percentage of cells in each phase of the cell cycle (S, G2, and M) was determined in duplicate.

DNA Analysis With PI and Bromodeoxyuridine.—After culture of HT-29 cells in nonarginine and 0.07-mmol/L and 0.57-mmol/L arginine-repleted RPMI medium, 10 mmol/L of 5′-bromo-2′deoxyuridine (BrdUrd) was added to selected cultures for 1 hour at 37°C. Cells were then fixed with ethanol and denatured with 4N hydrochloride with 0.5% trinitrotoluene (Triton-X 100, Sigma Chemical Corp). According to standardized procedures,[14] cells were then labeled with fluorescein isothiocyanate-conjugated anti-BrdUrd antibody (Becton Dickinson) followed by PI. Simultaneous red and green fluorescence was then measured as indexes of PI and BrdUrd incorporation, respectively.

Amino Acid Analysis

Analysis of amino acid in whole blood was performed by the Metabolic Assessment Laboratory of the University of Florida, Gainesville.

Statistical Analysis

Data regarding liver weights were expressed as means±SDs and analyzed for population differences by the two-tailed independent t test or by analysis of variance. Liver metastases were expressed as median values with associated ranges and analyzed with the Mann-Whitney U test.

RESULTS

Effect of Arginine Supplementation on the In Vivo Growth of Colorectal Liver Metastases

To determine the effect of the addition of arginine to the standard chow diet (which already contained 1.4% arginine), mice were randomly assigned to one of two groups before tumor cell inoculation. The first group was given water supplemented with 1% arginine hydrochloride ad libitum, whereas an isonitrogenous dose of 1.7% glycine was added to the water of the second group, which was allowed this water ad libitum. There was no significant difference between groups in water or chow intake. Similarly, although small splenic tumors were occasionally observed, splenic

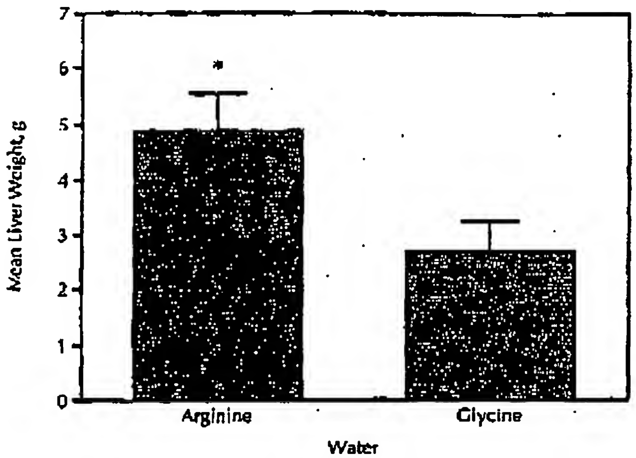

Fig 1.—Supplementation of water with 1% arginine (n=8) vs supplementation with 1.7% glycine (n=10) results in the stimulation of in vivo tumor growth (55% increase in weight; asterisk indicates P<.02) in the liver. Data are mean liver weights±SEs.

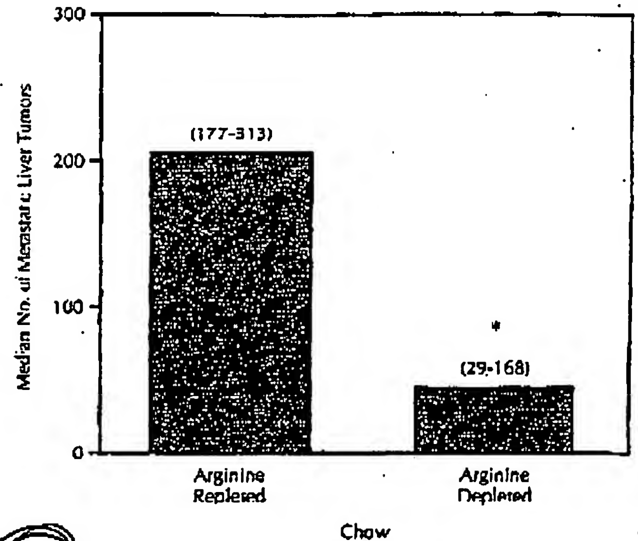

Fig 2.—Selective dietary depletion of arginine (n=8) vs arginine repletion (n=10) diminishes the growth of liver metastases by 78% (asterisk indicates P<.05). Ranges of counts of tumor colonies in the liver are displayed in parentheses.

weights between groups were not different.

Because metastases were confluent in both groups, livers were weighed as a measure of tumor burden with the finding that the mean liver weights for the group receiving water with arginine (4.9 g) were 55% greater ($P<.02$) than the mean weights of the group receiving water with glycine (2.7 g) (Fig 1).

Effect of Arginine Depletion on the In Vivo Growth of Colorectal Liver Metastases

To confirm the growth-dependence of metastatic tumors on dietary arginine, a second method of dietary arginine manipulation was used. Mice were randomly assigned to two groups and fed either an arginine-depleted (nonarginine), amino acid–defined diet or a standard-content arginine diet (1.2% arginine). In this experiment, tumor inoculum was reduced to 1.25×10^5 cells in 0.5 mL of phosphate-buffered saline to prevent confluence of metastatic foci. Again, no significant differences were noted between the two groups in water or chow intake or splenic weights. No splenic tumors were observed in these animals.

Arginine levels in whole blood were measured in animals (n=4) randomly selected from both groups. Although there was a trend toward decreased arginine levels in animals fed nonarginine diets (0.117±19 mmol/L) vs those fed arginine repleted diets (0.154±18 mmol/L), the difference was not significant ($P=.10$).

When the median number of metastases were ascertained for each group, the arginine-depleted group was found to have 78% fewer (median, 46 metastases per animal) grossly visible metastatic nodules than the arginine-repleted group (median, 206 metastases per animal; $P<.05$; Fig 2). The relative differences between the arginine-repleted and arginine-depleted groups can be seen in Fig 3. No significant differences were noted in liver weights between groups because of the smaller tumor burdens.

Effect of Arginine Depletion on Tumor Cell Growth In Vitro

Noting the apparent dependence of in vivo tumor growth on dietary arginine, the effects of arginine depletion and repletion were examined in vitro. CT-26 colon carcinoma cells (5×10^5) were cultured in duplicate in either depleted or repleted medium, with standard amounts of arginine (0.013%) being used in the latter. Nonarginine conditions essentially halted all cell growth, as evidenced by the retrieval (4 days after incubation) of 2.4×10^5 fewer cells than the number added. When the cells were cultured in the presence of arginine, there were 9×10^9 additional cells (total, 1.4×10^6; Fig 4). Viability was greater than 98% in both the arginine repleted and the arginine-depleted groups.

Flow cytometric analysis of DNA content/cell demonstrated that the difference between the groups might be secondary to effects on cell division. Growth-phase DNA analysis demonstrated that the percentage of cells in the S, G2, and M phases was significantly greater ($P<.02$) in the nonarginine-treated cells (32.5%) than in the arginine-treated cells (22.0%), suggesting that growth and progression through the cell cycle depend on arginine (Fig 5).

Further study of this cell cycle aberration was performed using human colon adenocarcinoma cells (HT-29). Tumor cell growth was significantly—but reversibly—inhibited in a dose-dependent fashion by selective arginine depletion from culture medium (Table 2). Note that the effective dose range in vitro (0.07 to 0.14 mmol/L) closely approximates the in vivo levels in whole blood (0.12 to 0.15 mmol/L). While HT-29 cells in cultures containing 0.14 mmol/L arginine or higher concentrations were recovered in numbers approximately four times that inoculated, only 44% of inoculated HT-29 cells were recovered from the nonarginine cultures. An intermediate number of cells (1.45 times the number inoculated) were recovered in the 0.07-mmol/L arginine cultures. Inhibition of tumor growth was unabated despite the addition of isonitrogenous concentrations of glycine. Tumor cells initially cultured in nonarginine medium and later recultured in 0.57-mmol/L arginine multiplied (2.12 times the num-

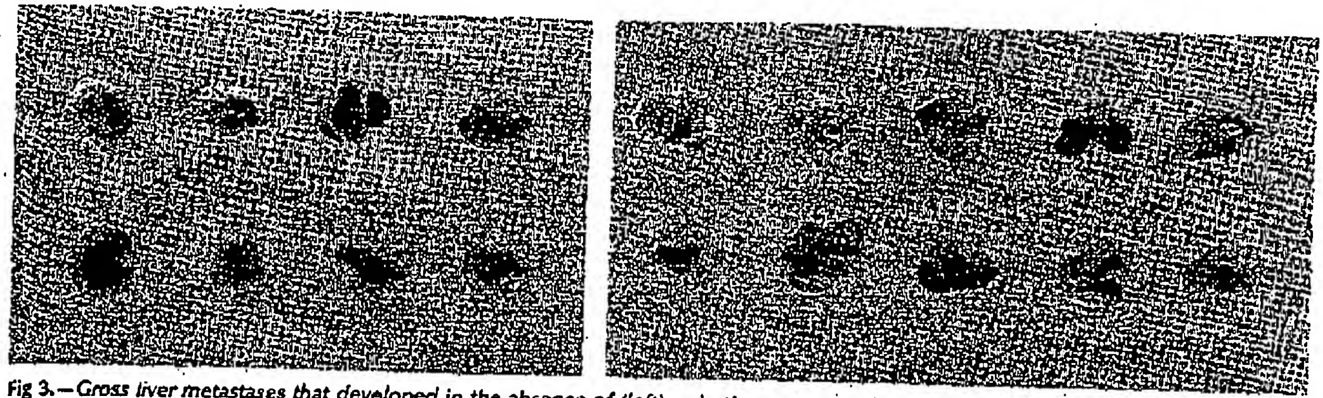

Fig 3.—Gross liver metastases that developed in the absence of (left) vs in the presence of (right) dietary arginine.

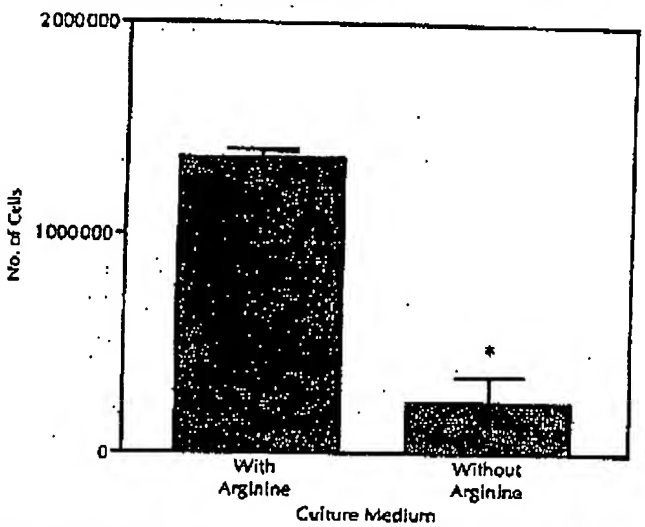

Fig 4.—Nonarginine cell culture medium results in less in vitro cell growth than does culture medium containing 0.013% arginine (n=2, asterisk indicates P<.02). Data are means±SEs.

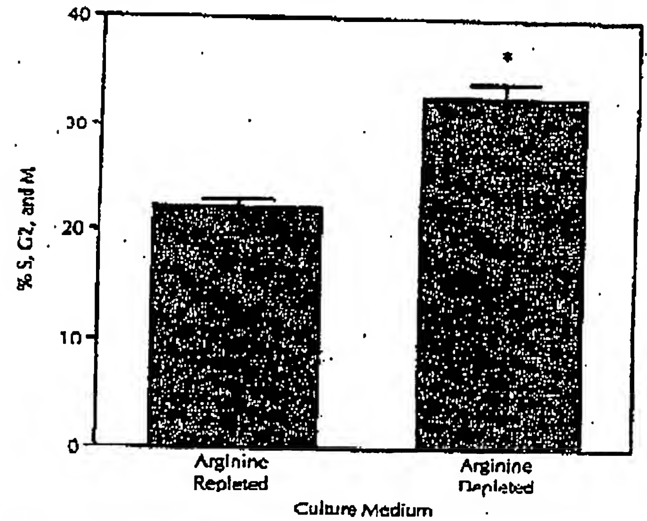

Fig 5.—Arginine depletion of cell culture medium in vitro, compared with arginine repletion, increases the percentage of cells in S, G2, and M phases of the cell cycle from 22.0% to 32.5% (n=2, asterisk indicates P<.02).

ber of cells inoculated were recovered), suggesting that the effect of arginine depletion is reversible. Flow-cytometric DNA analysis confirmed a progressive increase in the number of S-phase cells with decreasing arginine concentrations (ranging from 24% for 0.57-mmol/L arginine to 43% in the absence of arginine).

To determine the nature of this S-phase accumulation, cells were labeled with PI alone (controls) or with PI and BrdUrd simultaneously (Fig 6). The abscissa represents linear relative red fluorescence due to PI staining of cell nuclei. The ordinate represents log scale relative to green fluorescence secondary to BrdUrd uptake. While PI labels all DNA, BrdUrd competes with thymidine and labels only newly synthesized DNA and can be detected with fluorescein-labeled anti-BrdUrd monoclonal antibody.[15] These studies demonstrate that not all cells accumulating in S phase are actually synthesizing DNA but, rather, are quiescent. While nearly all S-phase cells grown in 0.57-mmol/L arginine were labeled with BrdUrd (Fig 6, C), fewer cells and almost none were labeled with BrdUrd when cultured in 0.07-mmol/L arginine (Fig 6, B) and nonarginine medium (Fig 6, A), respectively.

COMMENT

Using an advanced model of neoplastic disease, we found that dietary arginine depletion may reduce the growth of liver metastases. We also found that, using a weakly immunogenic tumor model, arginine supplementation may stimulate the growth of tumor in vivo.

Although the classic experiments of Rose et al[16] demonstrated that humans require only eight essential amino acids for nitrogen balance (isoleucine, leucine, lysine, methionine, phenlyalanine, threonine, tryptophan, and valine), Eagle[17] found that human and animal normal and neoplastic cells required additional amino acids for propagation in vitro. Arginine, cyst(e)ine, glutamine, histidine, and tyrosine were the additional amino acids identified. A number of theories have been proposed to explain why tumor can grow in vivo without the amino acids considered essential for in vitro culture conditions. One simplistic explanation is that the host may provide the arginine needed for tumor growth.

Despite some knowledge of the nature of amino acid growth requirements of tumor cells, both the role and mechanism of action of arginine in the tumor-host rela-

Table 2.—Dose-Dependent Effect of Arginine on Human Colon Cancer Growth In Vitro

	Arginine Concentration in Culture Medium, mmol/L*							
	None	0.07	0.14	0.29	0.57	1.14	4.50†	0.57‡
% cells recovered§	44	145	449	398	465	392	165	212
% G1-phase cells	47.9	51.6	None	None	59.5	None	None	53.6
% S-phase cells	43.3	33.9	None	None	24.4	None	None	35.6
% G2-phase and M-phase cells	8.9	14.5	None	None	16.1	None	None	10.8

*Cells were cultured in various concentrations of arginine for 3 days before harvest.
†Cells cultured in isonitrogenous glycine (control medium).
‡Cells were initially cultured in nonarginine medium for 3 days, then recultured in 0.57-mmol/L arginine for 3 days before harvest.
§Number of cells recovered divided by the number of cells inoculated and multiplied by 100. Viability was greater than 98% as measured by propidium iodide staining for all groups.

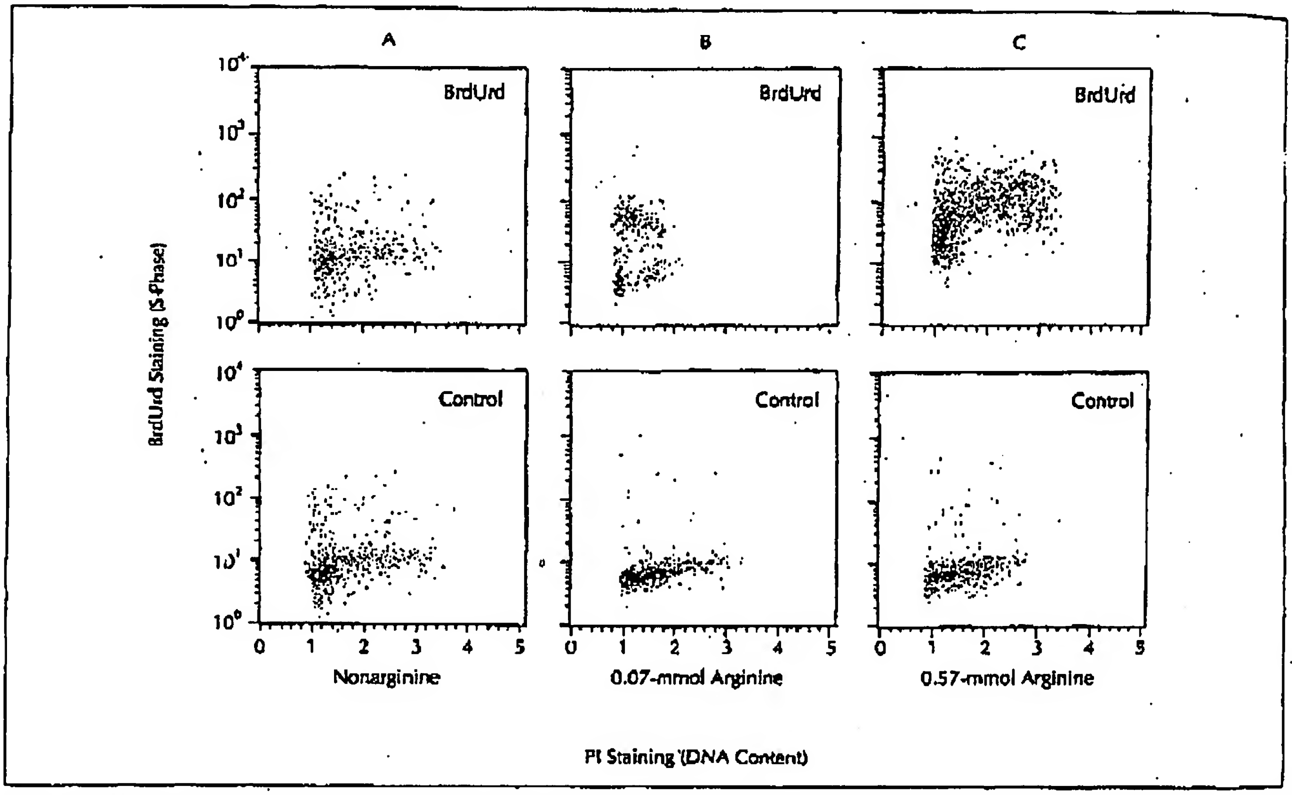

Fig 6.—Propidium iodide (PI) and 5'-bromo-2'deoxyuridine (BrdUrd)-labeled HT-29 cells. With arginine depletion (A and B), few S-phase cells (measured by PI uptake) synthesize DNA (measured by BrdUrd uptake), while most S-phase cells exposed to 0.57-mmol/L arginine (C) uptake BrdUrd.

tionship have not been elucidated. Although arginine appears to be an immunostimulant of cellular immunity in certain situations, it is not clear whether this effect applies only to immunogenic tumors. Reynolds et al[5] suggested that the antitumor effect of arginine may be mediated by arginine's modulation of host-tumor immune interaction, but only in tumors expressing immunogenic, tumor-associated antigens. In their model using protein-depleted mice, arginine suppressed the subcutaneous growth of moderately immunogenic tumor by enhancing cytotoxic T-lymphocyte development and natural killer cell activity while stimulating the growth of a poorly immunogenic clonal variant. Perhaps weakly immunogenic tumor escapes recognition and destruction by immune defenses—even when these defenses are augmented by supplemental arginine.

To define the effects of arginine on tumor growth (an issue separate from its potential immunostimulatory effects) we used a poorly immunogenic murine colon tumor with metastases introduced experimentally to the liver.[13] We hypothesized that in vivo tumor propagation might depend on the presence and quantity of arginine when propagation occurred while immune defenses may have been relatively ineffective. We chose a metastatic model of liver metastasis instead of the existing, common, subcutaneous-inoculation models because of its close approximation to human cancer progression.

We used two different in vivo experimental approaches, and the results of both experiments led us to the same conclusion: supplemented arginine enhances the growth of metastatic tumor cells, whereas its absence or deficiency inhibits growth. Differences in tumor growth

cannot be explained by the nutritional effects of arginine on liver mass. It has been reported[10] that supplementing water with arginine does not result in excessive changes in carcass weight compared with the carcass weight of control animals receiving water supplemented with glycine, and we found no differences in liver weights between the animals receiving arginine-depleted or arginine-repleted amino acid–defined diets. Differences in tumor growth appeared to be independent of nitrogen supplementation, as diets were isonitrogenous. Although these in vivo results could be interpreted as effects on tumor cell seeding efficiency (via effects on end-organ adhesion) rather than on the growth of tumor cells, we think this is unlikely because the tumor's growth dependence on arginine was also confirmed with in vitro experiments. There was no growth without arginine, but normal growth was observed when arginine was present in the medium. These results confirm that the original observations of Eagle[17] were also valid in our experimental model. This effect was similar for both murine (CT-26) and human (HT-29) colon tumor cell lines.

The apparent contradiction of a high S phase associated with lower but reversible growth rates in vitro is likely secondary to cells that have arrested in S_0 (the quiescent S phase)[18] and require arginine for complete cell cycle progression. Data from our experiments in which labeling of cells with PI and BrdUrd occurred simultaneously add supportive evidence to this hypothesis by demonstrating that a significant proportion of cells accumulating in the S phase secondary to arginine-depleted culture conditions are actually quiescent and do not synthesize DNA. Further study, perhaps using thymidine-pulse or 5-bromodeoxyuridine–pulse labeling,[14] is needed to determine the precise mechanism underlying these cell cycle aberrations.

These experimental findings suggest that metastatic tumor growth can be inhibited with dietary depletion of arginine. This decrease in tumor growth may be related to the basic tumor requirement of arginine for growth that was demonstrated in vitro and to the low level of tumor immunogenicity. Although this observation has yet to be made of cancer occurring in humans, potential clinical benefit might be obtained by using nonarginine hyperalimentation or amino acid–defined diets that may slow the growth of metastatic tumor. This concept is particularly relevant to humans in that most solid tumors are weakly immunogenic.

Additionally, because of the reversible nature of the in vitro accumulation of quiescent S-phase cells observed with arginine depletion, potential exists for the use of selective dietary arginine modulation (depletion followed by repletion) of cell cycle progression. For example, it might be possible to synchronize the growth of metastatic tumor cells in vivo and enhance the effectiveness of cell-cycle specific chemotherapy. Further studies examining the DNA from fresh tumor are needed to confirm this. Ultimately, we propose that arginine should not be considered "good" or "bad" for the tumor-bearing host, but, rather, viewed as a tool for modifying the biologic behavior characteristic of the tumor-host relationship.

We would like to thank David M. Ota, MD, for his thoughtful and constructive criticisms of this article and Philip Frost, MD, Departments of Cell Biology and Medicine, University of Texas, MD Anderson Cancer Center, Houston, for supplying the adenocarcinoma cells. We would also like to acknowledge the expert assistance of Nguyen Van, MD, in performing flow cytometric analysis.

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Discussion

DAVID M. OTA, MD, Houston, Tex: Have you looked at plasma arginine levels in your animals that were on the special diets? Please comment on a possible mechanism. Arginine seems to be an essential nutrient here. How do you think that impacts on the cell growth process within the tumor? Is it protein synthesis, or is it some other metabolic pathway for which arginine is essential for cell growth?

DAVID S. ROBINSON, MD, Miami, Fla: Can you tell us how those cells that escaped into G2 and M, still depleted of arginine, were allowed to get through? Then, with regard to the host, tell us about the remaining cellular tissues that are non-